HISTOLOGICAL AND HISTOCHEMICAL OF MUCUS PRODUCING CELL AT SEVERAL ORGANS OF PARROT FISH (*Scarus javanicus*)

Cahyono Purbomartono[1], Akihiro Takemura[2] and Kazunori Takano[2]

[1)] Department Biology, Faculty of Education, Muhammadiyah University of Purwokerto, Central Java, Indonesia, and [2)] Tropical Biosphere Research Center, University of The Ryukyus, Sesoko Station, Motobu-cho, Okinawa, Japan

Abstract

Mucosal secretion is the first line of defense and mucosal lining cells functions as the defensive cellular barrier against foreign agents. The mucus layer covering the body surface of teleost fishes is secreted by mucus cells (MC). In the present study, the structure of MC and its content in the epithelium of the buccal cavity, the gill arch, the esophagus and the skin of parrotfish (*Scarus javanicus*) were compared histologically and histochemically. A special mucus-secreting organ of the parrotfish (opercular gland) also observed using the same methods. Many MC distributed in the epithelium of buccal cavity, the esophagus and in the opercular gland of parrotfish. Many MC in the special gland of parrotfish suggest that these organs actively secrete much mucus. Contrary, lower density of MC was observed in the skin of parrotfish. Histochemical reactions revealed that out of eight lectin used in the present study. The WGA reacted widely with tissues of parrotfish in the epithelium of buccal cavity, opercular gland, gill and esophagus. These results suggests that N-'acetyl-D-glucosamine is a common carbohydrate residues of glycoproteins contained in mucus. Other lectins, PNA reacted with the skin of parrotfish. Mucus from these tissues contains carbohydrate residues of D-galactose for PNA. Mucus proteins containing these various carbohydrate residues may reveal characteristic natures of each tissues.

Keywords: histology, histochemistry, mucus, parrot fish

Introduction

Mucosal secretion is the first line of defense and mucosal lining cells function as the defensive cellular barrier against foreign agents (Dalmo *et al.,* 1997). The mucous layer covering the fish skin has been associated with several functions, such as abatement of turbulence generated by swimming, reduction of friction (Rosen and Comford, 1971), osmoregulation (Johnson, 1973) and resistance to fish pathogens (Manning 1994). Some of the less common properties offish cutaneous mucus are temporary shelter of potential predators (Winn, 1955), support for developing eggs, and feeding and attachment of the young.

Fish epidermis and its associated mucous layer is an immediate barrier to pathogens in the environment. Although antibodies can act as defense mechanism in fish skin, other anti-pathogenic substances such as complement, lysosomes, proteases and lectin-like molecules are present in skin and mucus (Alexander and Ingram 1992; Dalmo *et al*., 1997). Previous studies examining mucus secretion used predominantly histological techniques, involving staining and counting of mucous cells in defined surface areas (Pickering 1974, Pickering and Macey 1977, Pottinger *et al.* 1984, Urawa, 1992). Better understanding of the fish skin defense mechanism, mucus secretion being a major one, could be beneficial in improving fish health maintenance in aquaculture.

It has been also proposed that continuous secretion of the mucus prevents colonization of parasites, fungi, and bacteria (Pickering, 1974). In fact, some defense substances such as immunoglobulin (Rombout *et al*., 1995), lysozyme (Fletcher and White, 1973), C-reactive protein (CRP) (Ramos and Smith, 1978) and lectin (Itami *et al*., 1993; Suzuki, 1995) have been identified in the secreted mucus. Major constituents in mucus are glycoproteins which are secreted by mucous cells in body surface epithelium (Asakawa, 1970; Pickering, 1974). It is considered that there is large difference in the constituents among organ or tissues, on the basis of diversity of specific adaptability for environment in each species.

Chemical content of fish mucus consists

of free proteins, glycoproteins, nucleic acids, salts, and water, similar to the mucus secretion of other vartebrates (Creeth, 1978). Glycoproteins, a major component, give mucus its characteristic physico-chemical properties. Mucus is constantly secreted and shed with the microorganisms attached to it, providing a physical barrier (Manning, 1994). The amount of mucus secreted can be altered in response to stress (Ingram, 1980 and Manning, 1994).

Material and Methods

The fish used in the present study was parrotfish. Parrotfish was collected from the coral reef around the Sesoko island by a small-mesh casting net and maintained for several days in around-shape plastic tank (one metric ton capacity) with running seawater at the Sesoko Station, Trophical Biosphere Research Center, University of the Ryukyus, Motobu, Okinawa, Japan. The body length (SL) and body weight (BW) was 16.0-19.5 cm SL and 110-205 g BW.

After anaesthetizing the fish in 0.01% 2-phenoxyethanol (Kanto chemical, Tokyo, Japan), small pieces of the surface epithelium of opercular gland, buccal cavity, the primary lamella of the gill arch, the esophagus and the skin were taken from parrotfish (Fig. 1).

Fig. 1. Morphology of opercular gland of parrotfish. Arrow show the opercular gland

Histological procedures

Tissue samples were preserved in Bouin's solution for 24 hours at room temperature. They were dehydrated with serial concentrations of ethanol and embedded in histoparaffin (m.p. 56-58°C, Merck, Darmstadt, Germany). The embedded tissues were serially sectioned at 5 µm and stained with Mayer's haematoxylin-eosin. Observation of these tissues was done under a light microscope (Nikon, Alphaphot-2, Japan).

Histochemical procedures

Each tissue from the fish was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, and stored for several days at 4°C. They were embedded and sectioned as the above-mentioned methods. Lectins conjugated with FITC (Honen Corporation, Tokyo, Japan) were used for histochemical staining. The present staining procedures were as follows:

1) After deparaffinization with xylene and serial ethanol concentrations (100 to 70%), slides were washed three times with phosphate-buffered saline (PBS) pH7.4
2) The slides were incubated with FITC-lectins which were diluted with PBS (1:1000) at room temperature for 1 h under dark condition
3) They were washed three times with PBS.
4) They were mounted using 1,4-Diazabicyclo (2-2-2) octane solution (Sigma, St. Louis, MO) mixture with glycerol (1:1).
5) Observation was done under a florescent microscope (Nikon, Microphot-FXA, Japan).

Control slides were incubated with PBS without FITC-lectins at room temperature for 1 hour under dark condition.

Results and Discussion

Histological observations

In general, the skin consists of two layers, epidermis and dermis. The epidermis is superficial and epithelial in nature. The epidermis is composed several kinds of cells such as goblet/mucous cell, epithelial cell, and the cell of stratum germinativum. The dermis basically consists of a dense connective tissue enclosing the scales and the basement lamina below the scales.

The present observations focused on mucous-producing cells, mainly the mucous cells, in the skin. The mucous cells in the surface epithelium of inner cavity of several organs were also observed to make comparison of their structure and possible functions. Histochemical identification and characterization of epidermal mucous cells has been undertaken to supplement biochemical analyses of the epithelial mucins as a part of a detailed study of the biology of fish mucus.

The buccal cavity

Figure 2 shows cross sections of the surface epithelium of the buccal cavity, the opercular gland, the primary lamella of the gill arch, the esophagus and the skin of the parrotfish, Scarus javanicus. The surface epithelium of the buccal cavity was flat and in some portions showed a shallow depression (Fig. 2a). The epidermis consisted of mucous cells and stratified cuboidal epithelial cells. The mucous cells frequently accumulated mucus in the form of many vesicles, and distended to be termed goblet cells. Some of the goblet-like cells were observed to be opened to the outer surface. The number of goblet cells in this region was about 9 cells per 0.01 mm^2. The largest size of the goblet cell was 50 µm in lenght and 25 µm in width. The epithelial layer was supported by the connective tissue of the submucosa.

The opercular gland

The opercular gland of parrotfish was constructed of complicated invagination of an epithelial sheet. The epithelial layer with smooth surface was occupied by well developed goblet-like mucous cells and epithelial columnar cells which were arranged among the mucous cells. The mucous cells were spherical or cylindrical in shape, and distended with a large bulk of transparent inclusion. The flattened nuclei were found in the lower periphery of the goblet-like mucous cells. The number of goblet cells in this gland was about 26 cells per 0.01 mm^2 and the largest size was 45 µm in height and 40 µm in width. The submucosa consisted of loosely arranged, fibrous connective tissue (Fig. 2b).

The lamella of the gill

The epithelial layer of primary lamella of the gill arch includes several kinds of cells; chloride cells, pavement cells and mucous cells. In the apical portion of the primary lamella, the most peripheral layer of the epithelium were covered by squamous pavement cells and the moderately number of the mucous cells fronted to the free surface of this layer (Fig. 2c). The mucous cells were spherical and almost uniform in shape, measuring about 20 µm in diameter, and filled with mucous substance. The number of the mucous cells was about 9 cells per 0.01 mm2.

The esophagus

The esophagus is a muscular tube intervened between pharynx and stomach, and its structure is same as the digestive tube in general. The surface epithelium of esophagus of parrotfish formed numerous folds which showed the irregular form in cross section. The epithelial layer consisted of the goblet-like mucous cells and the epithelial columnar cells. The goblet-like mucous cells were arranged adjacently each other in a single layer. They, accumulated mucus in the cell center, showing spherical or cylindrical forms. The number of goblet cells were about 22 cells per 0.01 mm^2 in various size. The largest size was 40 µm in height and 35 µm in width (Fig. 2d).

The skin epithelium

The skin surface were covered with epidermis layer which consisted of the mucous cells and superficial epithelial cells. The mucous cells moderately developed and were interspersed among the superficial epithelial cells. They appeared to include

homogenous liquefied substance in the cell center. The number of mucous cells was few, about 5 cells per 0.01 mm^2. The largest size of 70 µm in height and 50 µm in width (Fig. 2e).

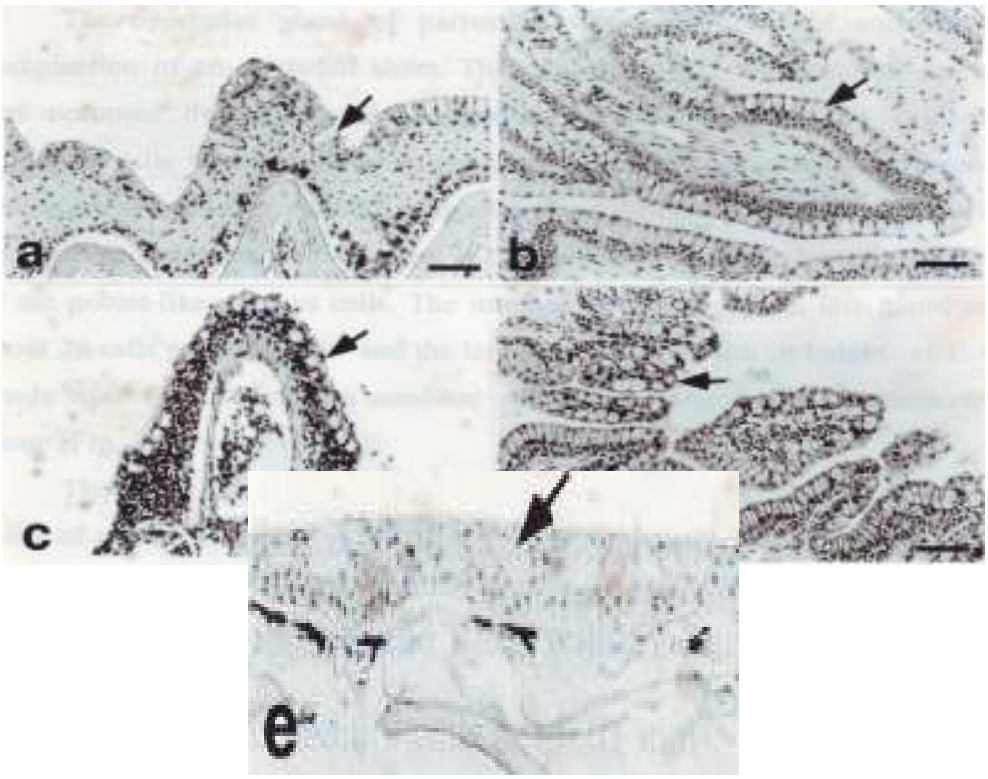

Fig. 2. Histological observations on several tissues of parrotfish (Scarus javanicus). Arrow show goblet cells in the: a, epithelium of buccal cavity; b, opercular gland; c, gill; d, esophagus and e, skin. Scale bars = 100 µm.

Histochemical Observations

Screening result of lectin staining

The epithelium of fish was stained with FITC-lectins. Of 8 lectins tested in the present study, WGA, PNA, and DBA showed positive reactions in the goblet cells of some tissues. WGA had positive reactions in the goblet cells of the surface epithelium of the buccal cavity, the primary lamella of the gill arch, the esophagus, and the opercular gland of parrotfish. The goblet cells of the skin from the parrotfish did not react with this lectin. PNA reaction appeared in the skin of parrotfish. Intensity of reaction with WGA, especially in the opercular gland, was much stronger than that with PNA. The goblet cells in the control sections were not stained.

Staining patterns of WGA, PNA and DBA-lectins

Staining patterns were observed in parrotfish using 8 kinds different lectin, and showed variation intensity from faint to strong. The strong and weak reactivity occurred in the parrotfish by the WGA and PNA. While, the faint reactivity also occurred in epidermis of buccal cavity by the WGA. The staining pattern of WGA were widely more reactive against several tissues and was followed by the PNA respectively. WGA showed strong reactivity with the goblet cells in the opercular gland of parrotfish (Fig.3), although various types of reaction from weak to strong were observed. Almost all of the goblet cells was stained with this lectin.

Mucus plays physical roles as protective barrier to external environments (Fishelson, 1996) and lubricant in aqueous

surroundings (West *et al.,* 1968), osmoregulation and locomotion (Cameron and Endean, 1973). It was showed that there is large difference in glycoprotein contents of mucus cells among teleost fishes (Harris and Hunt, 1973). Previous histochemical descriptions of such cells have shown that they contain both protein and carbohydrate (Asakawa, 1970).

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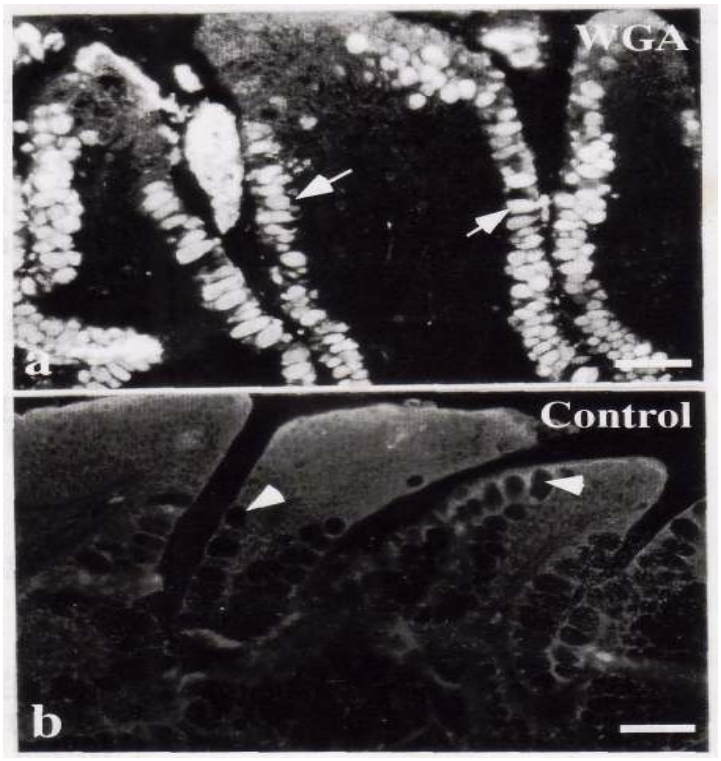

Fig. 3. FITC-lectin staining on opercular gland of parrotfish. a, opercular gland of parrotfish stained with WGA. Go blet cells were strongly stained in epidermis (arrow); b, opercular gland used as control. No reactions appeared in the goblet cells (arrowhead). Scale bars = 100 □m.

Proteins/peptides containing in mucus exhibit various pathological functions. Immunoglobulins (Takemura, 1994), lysozyme (Fletcher and White, 1973), C-reactive protein (CRP) (Ramos and Smith, 1978) and complements (Ingram, 1980) are known as a chemical barrier. Therefore, mucus may play a role in prevention of colonization by parasites, bacteria and fungi (Jakowska, 1963). It seems that there are other peculiar functions which exhibit by mucus itself. In the present study, peculiar functions of each organ were discussed from the comparison of number of mucus secreting cells in parrotfish *(Scarus javanicus).*

When compared with number of the goblet-like cells among several organ of parrotfish tested in the present study, the opercular gland, rather than other four tissues, had much more goblet-like cells in the surface epithelium. Moderate goblet cells were observed in the esophagus of parrotfish. Few goblet-like cells were observed in the surface epithelium of the buccal cavity of parrotfish. This difference in the number of the goblet cells in the digestive system may be partially due to feeding activity of the fishes. It was reported that suspended phytoplanktons are entrapped by copious quantities of mucus in the buccal cavity (Greenwood, 1953). In case of tilapia, Sanderson *et al.* (1996) reported, that mucus secretion rates are adjusted by response to particle size. Importance of mucus in the first part of digestive system would be necessary to study in connection with the feeding habit of this fish species. As mentioned later, however, it is possible that macromolecular

substances secreted from the epithelium play some role for digestion of this species.

The number of the goblet-like cells in the apical region of the primary lamella of gill was few relatively. The epithelium that surrounds the primary lamellae is compounded of several kinds of very specialized cells: pavement cells that make up the major surface of the gill, mucous goblet cells, chloride cells, accessory cells, non-differentiated supportive cells, basal cells and mast cells (Shimada *et al.* 1982). In such co-system of various type of cells, the mucous cells may play a major role in protection of surface by forming mucus barrier (McCahon *et al.,* 1987; Handy and Eddy, 1989). Moreover, it was suggested that the mucus film covering the secondary gill lamella in teleost fishes has an important function in gas, ionic and water changer at the gill surface (Hughes and Wright, 1970). Additionally the mucous goblet cells are particularly abundant in the gill primary lamella (Shimada *et al.,* 1982).

Parrotfish developed a moderate number of mucus secreting cells in their skin. This is thought as common characteristics among teleost fishes, body surface covered with much mucus exhibits various functions as mentioned above. On the contrary, rabbitfish had few goblet-like cells in the body surface. At present, meanings of the little of the mucus secreting cells on the body surface is still obscured, though it, is considered to produce other types of protective substances in this fish. This fish is well known to have special opercular gland which contains many goblet cells. They might be act as a defensive organ at least against predators (Cameron and Endean, 1973).

Parrotfish owns the specific tissue called as the opercular gland, from which mucus is secreted to cover body surface at night and become a cocoon. This gland consisted of complicatedly folded epithelial sheets, and many mucous cells distributed at the epidermis were recognized. This observation shows that secreting activity of this organ is very high. At present, the importance of the cocoon is not fully understood. Winn and Bardach (1959) suggested that the mucus envelopes produced at night by some parrotfishes reduce chemical conspicuousness. This may play a role in avoidance of predation. McCahon *et al.* (1987) and Handy and Edy (1989) reported that in such a system the mucous cells may play a major role in protection of surfaces by forming a barrier of mucus. These cells must adjust and modify themselves to respond to environmental variations (Playle and Wood, 1989). In this study, a provable relationship between the cocoon formation and changes in secretory activities in this gland was not ascertained from a preliminary rearing experiment in an aquarium.

In case of the opercular gland of the parrotfish, histologically and histochemically revealed many goblet-like cells were present in the epithelial layer. Physiologically, the mucous-like cells secrete mucus to protect body surfaces. Therefore, that opercular gland was presumed to be main gland/organ for producing mucus in parrotfish. Related to this fact, the cocoon was made by these mucus. Additionally, histological examination of the opercular gland showed, that there were not different morphology of each goblet-like cells between after and before secretion. It may the parrotfishes which were used in this present study, were maintained in the aquarium. With this captive condition, the parrotfish did not produce mucus properly. Whether naturally it is different between after and before secretion, or not, it may need further study.

Histochemical observations

Lectins are proteins which recognize specific carbohydrate structures and agglutinate various types of animal cells by binding cell-surface glycoconjugates. In the present study, eight lectins, WGA, PNA, LCA, RCA, PHA, Con-A, UAE and DBA were used as a tool for detection of glycoproteins in the mucus of three fishes. Out of eight lectins tested in the present study, WGA and PNA showed positive reactions. Reaction with WGA appeared in the surface epithelium of the buccal cavity, apical region of the primary lamella of gill, the esophagus and the opercular gland of parrotfish. Intensity of reaction were varied among tissues. As WGA lectin is specific for N-acetylglucosamine and/or sialic acid, this result suggests that such carbohydrate-residues binding glycoproteins exist commonly in the mucus of this fish. Gona (1979) observed that all the functions of mucus layer are closely related to the kind of glycoprotein produced by mucous cells. It has been recorded that WGA bound to the

goblet cells of the small and large intestines in rat and monkey, small intestine in human, and large intestine in rat and guinea pig (Madrid *et al.,* 1989). Furthermore, WGA was the lectin that showed the strongest affinity toward the intestinal microvilli in the above mentioned animals. These findings indicate wide distribution of GlcNAc and/or sialic acid residues in glycoconjugates of animals.

In the present study, PNA positive reactions appeared in the mucous cells of the skin of parrotfish. These results suggest that glycoproteins secreted from these sites have terminal residues of (□-galactose and □-N-acetylgalactosamine. The carbohydrate chains containing these sugars are considered to contribute viscoelastic barrier formation which protects the mucosa from the acid environment and proteolysis (Pajak and Danguy, 1993). The present histochemical observations revealed that distribution of the mucous cells reacting with these PNA was different from that of WGA. In the present study, it was not confirmed whether same mucous cells produce two distinct glycoporteins, WGA and PNA reacted ones, or not. However, it may be considered that the gylcoproteins binding with these two lectins, WGA and PNA, are related to specific functions of each site. Additionally, the avoidance to the predator was also assumed to affect the physiological condition. The parrotfish, a coral reef fish, has a special opercular gland in the body. At night time, the parrotfish can make a cocoon and cover whole body to avoid against predation.

Conclussion

Many mucous cell (MC) distributed in the epithelium of buccal cavity, the esophagus and in the opercular gland of parrotfish. In the special gland of parrotfish, many MC suggest that these organs actively secrete much mucus. Histochemical using lectin, the WGA reacted widely with tissues of parrotfish in the epithelium of buccal cavity, opercular gland, gill and esophagus. These results suggests that N-'acetyl-D-glucosamine is a common carbohydrate residues of glycoproteins contained in mucus. Other lectins, PNA reacted with the skin of parrotfish. Mucus from these tissues contains carbohydrate residues of D-galactose for PNA. Mucus proteins containing these various carbohydrate residues may reveal characteristic natures of each tissues.

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